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Filsoniana lhasanensis sp. nov. from Tibet, China

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Abstract—A new species, *Filsoniana lhasanensis*, is described from Tibet, China, where it grows on rocks at altitudes 4200–4432 m. The lichen is characterised by a poorly developed or absent dark orange areolate thallus, lecanorine to zeorine orange to deep-orange aggregated apothecia, and richly branched and anastomosed paraphyses. DNA was obtained for three gene loci—nuclear internal transcribed spacer (ITS), nuclear large subunit (LSU), and mitochondrial small subunit (SSU). Phylogenetic analyses support the taxon as a new species of *Filsoniana*.

Key words—Himalaya, phylogeny, taxonomy, *Teloschistoideae*

Introduction

Teloschistaceae is a widespread and well-delimited family of lichenized fungi, estimated at over 1000 species (Arup & al. 2013). Most species contain anthraquinone (which confers a yellow to orange color) and grow under sun-exposed conditions (Gaya & al. 2015). Recent molecular phylogenetic research supports subdivision of the *Teloschistaceae* into three subfamilies: *Xanthorioideae*, *Caloplacoideae*, and *Teloschistoideae* (Arup & al. 2013; Gaya & al. 2015). Kondratyuk & al. (2013, 2015), who evaluated *Teloschistoideae* based on three gene loci (ITS, LSU, SSU), proposed an additional subfamily *Brownlielloideae;* however this analysis was based on a hybrid sequence dataset comprising ITS and LSU data derived from

the lichen mycobiont and the SSU derived from a fungal contaminant (Vondrák & al. 2018). Many small monophyletic genera established in *Brownlielloideae* were based solely on DNA data without any distinguishing phenotypic characters, and these genera remain within the *Teloschistoideae* as proposed by Arup & al. (2013).

During our study on Chinese *Teloschistaceae*, we collected some interesting samples from the suburbs of Lhasa and Shannan in Tibet. The Tibetan plateau is a unique biogeographical region with varied landscapes, altitudinal belts, and alpine ecosystems and is considered a world center of species formation (Zhang & al. 2002). *Teloschistaceae* in this region have been inadequately studied, with only 15 species reported (Obermayer 2004; Wei 1991, 2017; Poelt & Petutschnig 1992; Poelt & Hinteregger 1993; Wei & Jiang 1986). More species are known from the neighboring Himalayan regions: India, where >74 species of *Teloschistaceae* have been recorded (Joshi & Upreti 2006, 2011; Joshi & al. 2008, 2009, 2014; Kondratyuk & al. 2018) and Nepal, where 47 species have been discovered (Olley & Sharma 2013). According to available literature, there are still large underexplored areas in the Tibet region, and additional undescribed lichenized fungi species are expected to be discovered in this poorly studied region.

Most of crustose *Teloschistaceae* species have few taxonomically significant morphological characters. Traditional species concepts based in morphology and chemistry are overly simplified for the crustose species in *Teloschistaceae*. Modern classification methods are essential for accurate taxonomic placement of rare and undescribed species. We combined sequence analyses of three gene loci—ITS, LSU, SSU—with morphological and chemical methods to describe a new species, *Filsoniana lhasanensis*. This is the first report of the genus *Filsoniana* in China.

Materials & methods

Morphology & chemistry

Specimens collected from Lhasa and Shannan, Tibet, China, were preserved in Tibet Plateau Institute of Biology, Lhasa, China (XZ) and Lichen Research Center in Arid Zone of Northwest China, Xinjiang University, Urumqi, China (XJU–L). The specimens were examined morphologically using a Shunyu SZM45 dissecting microscope and Nikon Eclipse E100 compound microscope. Colors follow RAL color system (https://www.ralcolorchart.com/) Sections for anatomical details and measurements were made manually and mounted in water. Chemical constituents were identified by thin-layer chromatography using solvent systems C (Orange & al. 2010). Thallus morphological structures were photographed with a Nikon

Table 1. Primers and cycling parameters used for PCR.

Locus	Primers	PCR settings
ITS	ITS1F (Gardes & Bruns 1993) ITS4 (White & al. 1990)	94°C–5 min; 6 cycles of: 94°C–45 sec, 57–52°C (decreasing 1°C per cycle) –55 sec, 72°C–1 min; 30 cycles of: 94°C–45 sec, 51°C–55sec, 72°C-1 min; and 72°C–7 min
LSU	AL1R (Döring & al. 2000) LR6 (Vilgalys & Hester 1990)	94°C–10 min; 6 cycles of: 94°C–1 min, 57–52°C (decreasing 1°C per cycle) –1 min, 72°C–1.2 min; 30 cycles of: 94°C–1 min, 51°C–1 min, 72°C–1.2 min; and 72°C–2 min
SSU	mrSSU1 (Zoller & al. 1999) mrSSU7 (Zhou & Stanosz 2001)	94°C–10 min; 6 cycles of: 94°C–45 sec, 57–52°C (decreasing 1°C per cycle) –55 sec, 72°C–1 min; 30 cycles of: 94°C–45 sec, 51°C–55sec, 72°C-1 min; and 72°C–7 min

DS-Fi2 Digital Camera using a Nikon MZ25 and Nikon Eclipse Ci stereomicroscope. All measurements were made using the Nikon Nis elements (D). Morphology and anatomy were described according to Vondrák & al. (2013).

DNA extraction, PCR amplification, DNA sequencing

Total genomic DNA was extracted using the Fungi Genomic DNA Extraction Kit, following manufacturer's instructions. Molecular data were generated for three loci: the nuclear internal transcribed spacer (ITS), nuclear large subunit (LSU), and mitochondrial small subunit (SSU). Primers and PCR cycling parameters used for amplification are summarized in Table 1.

Sequence analysis

The newly generated sequences were compared to GenBank database sequences using BLASTN search (http://www.ncbi.nlm.nih.gov/BLAST/). All sequences were aligned with sequences of selected representatives of *Teloschistoideae* obtained from GenBank (Table 2) using MAFFT version 7 (Katoh & Standley 2013) with G-INS-i method.

Phylogenies were generated using Bayesian inference and Maximum Likelihood (ML) methods. Bayesian inference used the best fitting model as inferred by jModel Test v2.1.6 (Darriba & al. 2012), implemented in MrBayes v3.2.6 (Ronquist & al. 2012) on the Cipres Scientific gateway (Miller & al. 2010), TrNef+I+G for ITS and LSU and HKY+I+G for SSU. Two parallel MCMCMC runs were executed, each using eight chains and 10,000,000 generations, with trees sampled every 1000th generation.

Maximum likelihood (RAxML) analyses were performed using RAxMLHPC v.8 on XSEDE (Stamatakis 2014) under the GTRGAMMA model on CIPRES Science Gateway (Miller & al. 2010). Rapid bootstrap analyses were performed with 1000 bootstrap replicates. The phylogenetic tree was drawn using FigTree ver. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 2. Sequences used in the phylogenetic analyses.
(Sequences generated for this study are in bold.)

Species	Voucher	ITS	LSU	SSU
*Brownliella aequa*ta	SK838 [4]	KF264627	KF264662	KF264688
	SK831 [4]	KF264626	KF264661	KF264687
B. kobeana	130318 [5]	KT456214	KT456229	KT456244
	120032 [5]	KT456212	KT456227	KT456242
B. montisfracti	SK231 [4]	KF264625	KF264660	KF264686
	SK230 [4]	KF264624	KF264659	—
Brownliella sp.	SK2013a [4]	KF264628	KF264663	—
Caloplaca sp.	Casan 242 [3]	KT291463	KT291558	KT291508
	Gaya 110 [3]	KT291456	KT291552	KT291499
Elixjohnia bermaguiana	Kondratyuk 20487 [1]	KC179299	KC179245	KC179584
E. gallowayi	Kärnefelt 997504 [1]	KC179301	KC179247	KC179586
E. jackelixii	U1402 [1]	KC179303	KC179248	KC179587
	SK910 [4]	KF264655	KF264683	KF264707
Filsoniana australiensis	SK851 [4]	KF264631	KF264665	KF264691
	SK850 [4]	KF264632	KF264666	KF264692
F. lhasanensis	**Z24, Wen 20110910-05**	**MK153161**	**MK439835**	**MK439831**
	Z19, Wen 20110901-29	**MK153160**	**MK439834**	**MK439830**
*F. rexfilsoni*ï	SK859 [4]	KF264638	KF264671	—
	SK861 [4]	KF264636	KF264670	—
Follmannia orthoclada	Frödén 1772 [1]	KC179291	KC179191	—
Haloplaca sp.	6-UA2013 [1]	KC179295	KC179203	KC179537
Harusavskia elenkinianoides	SK997 [6]	KY614404	KY614452	KY614485
	SK269 [6]	KY614405	KY614453	KY614486
Josefpoeltia parva	Frödén 1671 [1]	KC179296	KC179204	KC179539
J. sorediosa	Frödén 1593 [1]	KC179297	KC179205	KC179540
	SK992 [4]	KF264646	KF264674	KF264697
	SK991 [4]	KF264645	KF264673	KF264696
Kaernefia albocrenulata	SK245 [4]	KF264647	KF264675	KF264698
	SK246 [4]	KF264648	KF264676	KF264699
K. gilfillaniorum	SK999 [4]	KF264650	KF264678	KF264701
	SK253 [4]	KF264649	KF264677	KF264700
K. kaernefeltii	SK919 [4]	KF264651	KF264679	KF264702
	SK921 [4]	KF264652	KF264680	KF264703
Nevilleiella lateritia	SK 261 [6]	KY614427	KY614464	KY614502

Species	Voucher	ITS	LSU	SSU
	SK 878 [6]	KY614426	KY614463	KY614501
N. marchantii	SK D18 [6]	KY614425	KY614462	KY614500
Scutaria andina	PF209 [1]	KC179298	KC179242	KC179581
Sirenophila cliffwetmorei	SK A93 [6]	KY614438	KY614471	KY614513
S. eos	U1408 [1]	KC179300	KC179246	KC179585
S. gintarasii	SK D17 [6]	KY614437	KY614470	KY614512
S. maccarthyi	Karnefelt 977801 [1]	KC179304	KC179249	KC179588
Solitaria chrysophthalma	Cchr157 [3]	KT291446	KT291537	KT291484
Stellarangia elegantissima	PF 81 [1]	KC179310	KC179254	KC179593
	Cele 75 [3]	KT291454	KT291541	KT291488
Teloschistes exilis	Gaya 12 [2]	JQ301630	JQ301577	JQ301519
T. flavicans	AFTOL-ID 315 [2]	JQ301578	JQ301631	JQ301520
	Tflav103 [3]	KT291472	KT291565	KT291523
T. hosseusianus	Gaya 50 [2]	KC179318	JQ301579	JQ301521
T. hypoglaucus	PF68 [1]	KC179319	KC179256	KC179595
T. sieberianus	Tsie423 [3]	EU639655	—	KT291525
Teloschistopsis bonae-spei	PF82 [1]	KC179322	KC179257	KC179596
Ts. eudoxa	PF72 [1]	KC179324	KC179258	KC179597
Thelliana pseudokiamae	SK925 [4]	KF264633	KF264667	—
	SK926 [4]	KF264634	KF264668	—
	SK 925 [5]	KT456225	KT456240	—
	SK 926 [5]	KT456226	KT456241	—
Villophora isidioclada	USE563 [1]	KC179325	KC179266	KC179606
Villophora sp. 50	U1342 [1]	KC179330	KC179268	—
Villophora sp. 40	USE574 [1]	KC179328	KC179267	KC179607
Wetmoreana decipioides	Cadec188 [3]	KT291453	KT291540	KT291487
	SK689 [4]	KF264644	—	KF264695
W. texana	SK536 [4]	KF264658	—	KF264711
	USE527 [1]	KC179337	KC179273	KC179612
W. sp. 52	Frödén 1645 [1]	KC179334	KC179270	KC179609
W. sp. 54	Frödén 1521 [1]	KC179336	KC179272	KC179611
Xanthomendoza fallax	Gaya 33 [2]	JQ301687	JQ301580	JQ301633
Xa. ulophyllodes	**T11, Shahidin 145507**	**MK414784**	**MK439836**	**MK439832**
	T22, Shahidin 145449	**MK414785**	**MK439837**	**MK439833**
Xanthoria sp.	Gaya 31 [2]	JQ301692	JQ301591	JQ301532

Sources: [1] Arup & al. (2013); [2] Gaya & al. (2012); [3] Gaya & al. (2015);
[4] Kondratyuk & al. (2013); [5] Kondratyuk & al. (2015); [6] Kondratyuk & al. (2017).

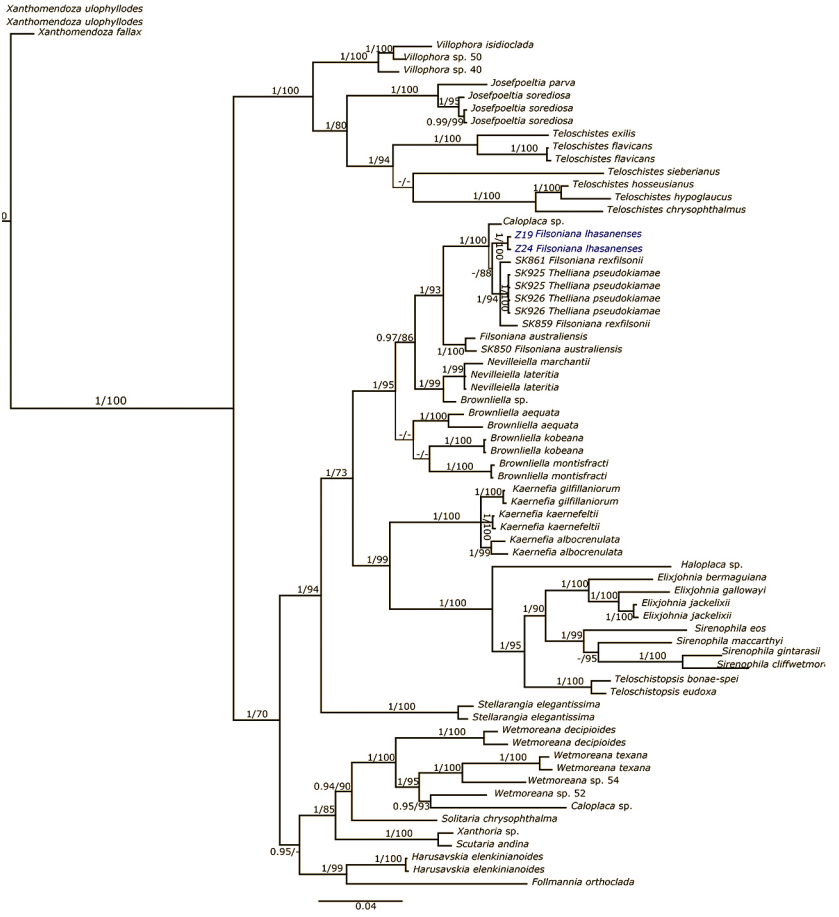

Fig. 1. The ML tree based on a concatenated 3-loci data matrix. The numbers above each node represent posterior probability (PP) and bootstrap support (BS) values. Only PP values >0.90 and BS values >70% are shown. The species described in this paper is shown in blue font.

Results & discussion

New ITS, LSU, and SSU sequences were generated in this study. A total of 70 ITS sequences, 67 LSU sequences, and 58 SSU sequences were included in the analyses (Fig. 1). Trees of similar topologies were also generated using the maximum likelihood method and Bayesian approach,

with *Xanthomendoza ulophyllodes* and *X. fallax* as outgroup. Our combined analyses grouped our new species with *Filsoniana rexfilsonii* and *Thelliana pseudokiamae* in a monophyletic clade with high support value (BS = 93, PP = 1). The new species is closely related to *Thelliana pseudokiamae* (S.Y. Kondr. & Kärnefelt) S.Y. Kondr.& al. and *F. rexfilsonii* (S.Y. Kondr. & Kärnefelt) S.Y. Kondr. & al.. Almost all other members of *Filsoniana* have well-developed thalli and occur in Australia. The sequences listed by Kondratyuk & al. (2013) under the name "*Squamulea kiamae*" were misdetermined; they were derived from specimen Kärnefelt 994101 [LD 1101337], which was resequenced by Kondratyuk & al. (2015) and designated as the holotype of a new genus and species, *Thelliana pseudokiamae* S.Y. Kondr. & al. Therefore all four of the "*Squamulea kiamae*" sequences represent *Th. pseudokiamae,* and authentic sequences of *S. kiamae* are not included in our analyses. *Filsoniana kiamae* and *Th. pseudokiamae* differ in the sorelia and lobe size (Kondratyuk & al. 2007, 2015). Our results conflict with Kondratyuk & al. (2015), whose ITS–LSU–SSU combined analyses placed *Filsoniana* in *Brownlielloideae;* our analyses place most members of *Brownlielloideae* in the *Teloschistoideae*.

Taxonomy

Filsoniana lhasanensis X.M. Wen, Shahidin & A. Abbas **sp. nov.** Fig. 2

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Differs from *Filsoniana kiamae* and *F. rexfilsonii* by its reduced thallus, its aggregated adnate apothecia, and its Asian distribution.

Type: China. Tibet, Lhasa City, Maizhuokunggar county, Riduo Village, 29°42′35″N 92°05′10″E, alt. 4200 m, 1 September 2011, X. M. Wen 20110901–29 (**Holotype**, XZ; **isotype**, XJU–L; GenBank MK153160, MK439834, MK43983).

Etymology: The epithet *lhasanensis* refers to the region in which the type specimen was collected.

Thallus crustose, areolate, poorly developed or absent, usually confined to the apothecia, 0.1–0.2 mm diam., orange to deep orange [RAL 2011]. Cortex paraplectenchymatous, 12.9–67.1 µm high, composed of thin-walled cells, (2.38–)4.8–7.4(–10.5) µm diam. Algal layer ± continuous, 49.5–97.9 µm thick. Medulla loosely interwoven by thin-walled hyphae under the apothecia, 59.8–86.1 µm high.

Apothecia lecanorine to zeorine, numerous, adnate, always aggregated (2–7) or crowded together, disc margins present, smooth, entire, concolorous with thallus, sun yellow [RAL 1037] to deep orange

[RAL 2011], disc round to irregular, yellow-orange [RAL 2000] to signal orange [RAL 2010], flat to concave, 0.3–0.7 mm diam. Exciple 9.6–14.8 µm thick, consisting of 2–4 layers of cells, upper part of cells spherical, 3.2–6.0 µm diam, cells of lower part elongated, 3.5–10.6 × 1.4–3.6 µm. Hypothecium thin, 22.3–34.7 µm high, composed of several lines of paraplectenchymatous cells, 2.9–5.2 × 2.3–4.6 µm. Hymenium 52.2–89.5 µm high, colorless. Paraphyses anastomosed, generally dichotomously branched or trigeminally branched at tips, the 3–4 terminal cells gradually enlarging towards tips, the widened cells nonuniform in size, the second cells 3.5–6.7 µm diam., and the third ones 3.2–5.3 µm diam.

Asci teloschistes-type, 8-spored. Ascospores colorless, ellipsoid, (11.2–)11.7–17.5(–4.7) × 5.8–8.4(–9.3) µm, septum 3.3–6.5 µm diam.

Spot tests—K+ red.

Chemistry—Parietin, emodin, and two unknown anthraquinones.

Ecology—*Filsoniana lhasanensis* grows on rocks in open areas and associates with species of *Aspicilia, Lecanora, Rusavskia, Physcia, Rinodina, Rhizoplaca,* and *Acarospora*. The new lichen is distributed in arid or semiarid areas of Lhasa city and Shannan city at elevations of 4200–4432 m.

Additional specimen examined: **CHINA. Tibet**, **Shannan City**, Nagarze county, Yamzho Yumco, 29°09′34″N 90°30′19″E, alt. 4432 m, 10 September 2011, X.M. Wen 20110910–05 (XZ; GenBank MK153161, MK439835, MK439831).

Remarks: *Filsoniana lhasanensis* is characterized by a reduced, dark orange to brownish orange thallus and aggregated apothecia. It is morphologically similar to *Xanthocarpia ferrarii* (Bagl.) Frödén & al., which differs by its narrower septum, larger ascospores, well developed true exciple, zeorine to biatorine apothecia, and classification in *Xanthorioideae* (Arup & al. 2013).

Filsoniana australiensis, F. rexfilsonii, and *F. lhasanensis* have similar paraplectenchymatous cortex and broad paraphyses (Kondratyuk & al. 2007). However, *F. australiensis* and *F. rexfilsonii* have well-developed thalli (*F. australiensis* has a placodioid thallus, and *F. rexfilsonii* has a squamulose thallus) with scattered or rare apothecia, whereas *F. lhasanensis* has a poorly developed thallus consisting of only a few scattered areoles.

A summary of the characteristics of *F. lhasanensis* and morphologically similar species is provided in Table 3.

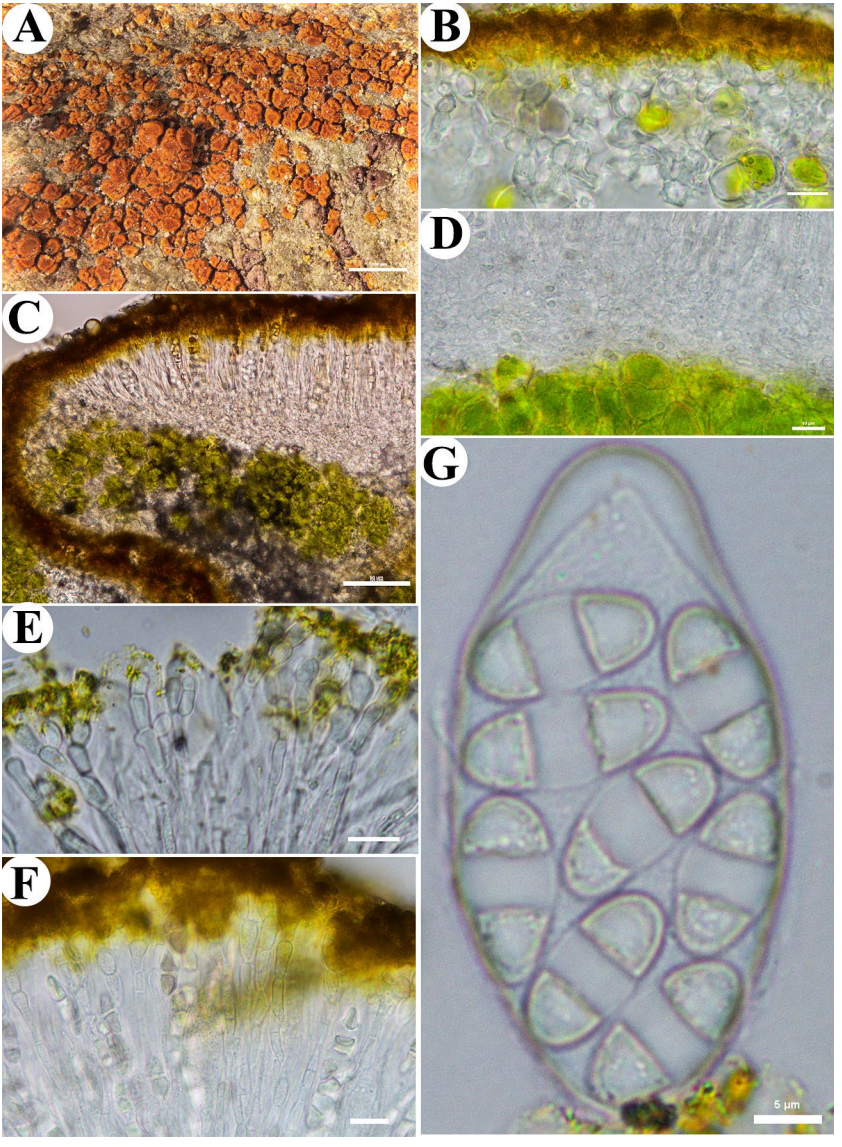

Fig. 2. ***Filsoniana lhasanensis*** (XZ (Wen 20110901–29)): A. Thallus and apothecia; B. Cortex of thallus; C. Cross section of the apothecium; D. Hypothecium; E. Paraphyses branched and enlarged at the tips; F. Paraphyses branched and anastomosed; G. Ascus containing eight ascospores. Scale bars: A = 1 mm; B–F = 10 µm; G = 5 µm.

Table 3. Comparison of *Filsoniana lhasanensis* with morphologically similar species of *Filsoniana*, *Thelliana*, and *Xanthocarpia*

Character	*F. australiensis*	*F. rexfilsonii*	*X. ferrarii*	*T. pseudokiamae*	***F. lhasanensis***
Thallus	Well developed, placodioid, distinctly lobate, dull pink to dirty brownish pink	Well developed, squamulose, brownish orange	Absent or poorly developed, pale gray	Margins, brownish yellow, with rare brighter yellow blastidia or punctiform sorelia	Absent or poorly developed, usually restricted around apothecia, orange to deep orange
Apothecia	Mainly lecanorine, sometimes zeorine, immersed in papillae to sessile, disc concave, reddish orange	Zeorine, margin slightly raised, disc dark orange	Common, zeorine to biatorine, orange to dark orange	Biatorine, rare, disc & proper margin reddish orange	Lecanorine to zeorine, numerous, adnate, aggregated, disc yellow-orange to signal orange
Paraphyses	Rather wide, simple, apical cell 4–5 µm	Apically widened, apical cells 6–7 µm, with oil droplets	Simple to branched, apical cells 4–5 µm	Not observed	Anastomosed, tips branching, the 3–4 terminal cells gradually enlarged towards the tips
Ascospores	10–13 × 6–8 µm	16–20 × 6.5–7 µm	17–23 × 5.5–7.5 µm	Not seen	11.7 –17.5 × 5.8–8.4 µm
Septal diam.	4–6 µm	2–5 µm	1–2 µm	Not observed	3.3–6.5 µm.
Ecology	Exposed rocks	In sheltered or exposed situations, on various rocks	Rocks	Rock surfaces or in rock crevices	Rocks in open areas
Distribution	Australia	Australia	Europe–central Asia	Australia	China

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